instead of glycogen for reasons given above.

Preliminary studies on the efficiency of the glycolytic cycle in the livers of normal and dystrophic mice fail to suggest a defect.

The activity of a number of enzymes of normal and dystrophic human muscle, including some operative in the glycolytic and citric acid cycles, has been appraised by histochemical technics. No significant differences were reported(12).

Suspensions of normal and dystrophic muscle mitochondria appear to respire at about the same rate. The intactness of the cycle has also been verified by Baker(13), who found the mean value of carbon dioxide production per gram of body weight equal in normal and dystrophic mice. This suggests that both oxygen consumption and carbon dioxide output are proceeding in an orderly fashion in all cycles tested. The respiratory rate is slow in both normal and abnormal muscle homogenates.

Preliminary experiments with liver homogenates of normal and dystrophic mice also fail to suggest significant differences in the respiratory rates.

Summary. The intactness of the glycolytic cycle was evaluated in homogenates of dystrophic mouse muscle, and contrasted with their normal littermates. The cycle, as measured by lactic acid production, appeared to operate efficiently. Similarly, the citric acid cycle was tested by measuring O_2 consumption of mitochondrial and microsomal suspensions derived from normal and abnormal mouse muscle. This cycle also seemed intact. Preliminary experiments with liver homogenates suggested that both cycles were unimpaired in dystrophic mice.

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Inductive Factors in Gliosis.* (27822)

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Recent work utilizing morphologic histochemical methods has indicated that a marked and sustained increase in activity of a number of oxidative enzymes occurs in reactive glia(1,2,3). There is a resemblance between this phenomena and that of adaptive enzyme synthesis in bacteria(4), as well as inductive enzyme synthesis in mammalian liver(5,6). Accordingly, a search for a possible inducing factor or factors responsible for this increase in enzyme activity as well as the reactive gliosis itself would appear warranted. Two questions which immediately arise are: (1) does

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the postulated inducing substance arise from within the cell or immediate environment or is it transported to the reactive cells *via* the circulatory system, and (2) the nature of this substance or substances. Previous authors have suggested a variety of stimuli which might be responsible for the transformation of resting glial cells into their reactive counterpart: partial tissue anoxia, products of altered cell metabolism, substances liberated by cell necrosis and invasion by inflammatory cells (7). The possibility that substances from the circulating blood might gain entrance *via* a damaged blood-brain barrier has also been considered(3). This latter mechanism appeared to offer a particularly fruitful field for investigation since blood-brain barrier alteration has been well described in many lesions productive of reactive gliosis(8,9,10). Our study, however, will demonstrate that the increased enzymatic activity of reactive glia is independent of alterations in the blood-brain barrier.

Methods. Male Holtzman Sprague-Dawley rats, 5 to 6 months old, weighing 400 to 500 g, were employed. The spinal cord was transected immediately above the lumbar enlargement and animals were examined at 24, 48, 72 and 96 hours after production of the lesion. Two hours prior to sacrifice each animal was injected intravenously with sodium fluorescein 0.2 mg/g body weight. Following sacrifice specimens were taken from the upper cervical cord and from an area immediately adjacent to the site of transection. Fresh specimens were examined under ultraviolet illumination, then placed on block holders and quick frozen in pulverized dry ice. Sections were cut at 16 μ in a cryostat, mounted on coverslips, thawed at room temperature and air dried. Enzymatic histochemical studies for demonstration of diphosphopyridine nucleotide (DPNH) diaphorase and triphosphopyridine nucleotide (TPNH) diaphorase were carried out utilizing 2,2′-di-p-nitrophenyl - 5,5′ - diphenyl - 3,3′ - (3,3′ - dimethoxy - 4,4′ - biphenylene) ditetrazolium chloride (Nitro-BT), 0.5 mg/ml, and reduced nucleotide, 1.5 mg/ml made up in modified Kreb's solution buffered at pH 7.4. Control sections were incubated in media lacking the substrate. Following 10 minutes incubation sections were fixed in buffered formalin, washed and either mounted in glycerine or dehydrated and mounted in Permount. Adjacent sections were formalin fixed for 10 minutes and stained with gallocyanin-chromalum at pH 1.8 directly, or following incubation for 30 minutes in a solution containing ribonuclease (RNAase) 1 mg/ml. Other sections were examined in the uncovered dry state by ultraviolet microscopy. H & E sections were also prepared from each tissue block. With sections prepared from the upper cervical cord, cell studies and comparisons were performed on the posterior columns. Utilization of this region presented an advantage in that unaltered tracts having their origin above the level of transection were sharply demarcated from the degenerating fibers due to the well known lamination of the tracts in this area, thus serving as an internal control in each animal.

Results are summarized in Table I. Initial morphologic evidence of glial reaction in the degenerating area of the dorsal funiculus occurred 48 hours following cord transection, and consisted of a slight enlargement of glial cell cytoplasm. Progressive cytoplasmic enlargement and demonstration of cell processes occurred in the following 48 hours. Early microglial rod forms were evident by 96 hours. Glial RNA was definitely increased at 48 hours, and at 96 hours the reactive cells contained large quantities of gallocyanin positive-ribonuclease sensitive material (Fig. 3, 4). TPNH diaphorase activity remained at levels seen in normal glial cells until 24 hours following the onset of RNA accumulation at which time a marked and relatively sudden increase occurred (Fig. 1,2). A slight increase in DPNH diaphorase activity occurred at the time of alteration in TPNH diaphorase, but was at no time as active as TPNH diaphorase.

Extensive alteration in the blood spinal cord barrier was demonstrated at the site of cord transection. Spinal cord tissue was brightly fluorescent when examined grossly by ultraviolet radiation as well as when examined with the fluorescence microscope. In the extensively altered areas of the cervical

TABLE I. Glial and Blood-Spinal Cord Barrier Alterations Following Cord Transection.

	Degenerating area cervical posterior column				Transection site
Age of lesion	Glial RNA*	Glial TPNH diaphorase†	Glial DPNH diaphorase†	Fluorescence‡	Fluorescence
Normal control	1+	1+	1+	0	—
24 hr	1+	1+	1+	0	4+
48 "	2+	1+	1+	0	4+
72 "	5+	4+	2+	0	4+
96 "	5+	5+	3+	0	4+

* RNA estimated by RNAase sensitive, gallocyanin-chromalum positive cytoplasmic material: 5+ (maximum), dark grey, markedly enlarged cytoplasm; 2+, light grey, slight cytoplasmic enlargement; 1+, very light grey without cytoplasmic enlargement.

† Enzyme activity estimated by formazan deposition after 10 min incubation. 5+ (maximum formazan deposition), very deep blue; 4+ to 1+, progressively lighter blue.

‡ Fluorescence in ultraviolet light following intravenous fluorescein: 4+, bright yellow-green fluorescence; 0, pale blue fluorescence of normal white matter.

posterior column no unusual fluorescence was detected grossly or microscopically at any time in any animal.

Although the observations reported here refer to glial changes in areas of the posterior columns undergoing Wallerian degeneration, alterations were seen in other areas of the cervical cord. Simultaneous with glial alterations in the posterior column, similar but less marked changes were seen in areas containing other ascending tracts. Since ascending tracts other than those in the dorsal funiculus are loosely grouped and partly admixed with other fiber systems, direct comparison with adjacent uninvolved tissue was extremely difficult.

Discussion. These results clearly demonstrate that the morphologic and enzymatic changes of early gliosis may be produced in the absence of demonstrable blood-spinal cord barrier alteration. These findings suggest that agents inducing gliosis or precursors of the agents may be pre-existent in normal central nervous system tissue. Due to the peculiar anatomy of the ascending tracts of the posterior column a number of pre-existent cytologic and cytochemical factors can be eliminated from consideration in the search for inducing agents. Desoxyribonucleic acid and products of desoxyribonucleic acid breakdown are not liberated at or near the point of glial reaction since only cell processes are undergoing degeneration. Similarly, products liberated by the destruction of nerve cell bodies and glial elements may be eliminated since such changes are far removed from the site of reaction. Infiltrative cellular elements, such as polymorphonuclear leukocytes, are not present within the area of reaction and appear to be unassociated with the cellular changes.

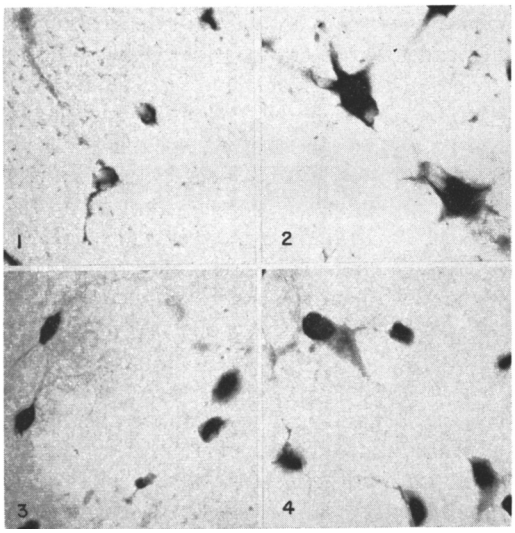

FIG. 1. Normal cervical posterior column. TPNH diaphorase. Formazan deposits appear black or a darker grey than the background. Two glial cells contain a moderate formazan deposit within cell body and processes. ×625.

FIG. 2. Cervical posterior column in area showing early Wallerian degeneration 96 hr after cord transection. TPNH diaphorase. A very dense formazan deposit is contained in 2 reactive cells. ×625.

FIG. 3. Normal cervical posterior column. Gallocyanin-chromalum. A very small amount of basophilic cytoplasmic material can be seen in some glial cells. This cytoplasmic basophilia is destroyed by RNAase treatment. ×625.

FIG. 4. Cervical posterior column in area showing early Wallerian degeneration 96 hr following cord transection. Gallocyanin-chromalum. Many glial cells demonstrate a marked increase in RNAase sensitive cytoplasmic material. ×625.

Conclusion. Morphologic and histochemical studies of glial reaction during Wallerian degeneration in the dorsal column of the spinal cord have shown that reactive gliosis is independent of alterations of the blood-spinal cord barrier. These data suggest that substances inducing gliosis may pre-exist in normal central nervous system tissue.

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Influence of Pregnancy and Cortisone Treatment on Brown Adipose Tissue in Bats.* (27823)

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The demonstration of rabies virus in the brown adipose tissue of experimentally and naturally infected Chiroptera has led to the hypothesis that this tissue, the so-called hibernating gland, may serve as an extraneural site for viral sequestration in the naturally infected, asymptomatic bat(1,2). Subsequent studies have dealt with the influence of certain stress factors which the bat may encounter or experience in nature (*i.e.*, environmental temperature and the reproductive cycle) on the course of rabies infection in these animals(3,4). Although the frequency with which bats could be experimentally infected with rabies virus was not increased during the gestation period over that observed in the non-gravid animal, the ability to demonstrate viral proliferation in the brown fat of infected gravid animals varied noticeably with the stage of pregnancy(4). During the course of these studies differences were noted in gross appearance of the brown fat lobes of bats in various stages of the gestation period, and the amount of tissue present in animals at time of delivery and in the early postpartum period was depleted. In view of these findings, the present study was undertaken to examine histologically the brown adipose tissue of the Mexican free-tailed bat (*Tadarida b. mexicana*) throughout the reproductive cycle. In addition, since cortisone has been shown to greatly influence the brown fat of hamsters and mice(5) and of rats(6), the response of this tissue in cortisone treated, non-gravid bats is presented.

Methods. Spring-breeding Mexican free-tailed bats,† *Tadarida b. mexicana,* used throughout these studies, were maintained at 29°C ± 2°C (relative humidity 60-70%). Methods for the laboratory care and maintenance of these animals have been described (7).

Gravid bats were netted at progressive intervals in the reproductive cycle between early May and late June. In groups of 25-30 per

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† Bats were obtained from the Blowout Cave, Blanco Co., Texas.